P- SOLUBILIZING MICROORGANISMS PERFORMANCE ON MANURE AND ROCK PHOSPHATE AND THEIR INFLUENCES ON SOIL AND PLANT PHOSPHOROUS IN CALCAREOUS SOILS

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ABSTRACT

Phosphate solubilizing microorganisms were isolated in vitro experiment, and wheat crop were planted in pots for 5 month and treated with rock phosphate, manure and with combination treatments of rock phosphate, manure, and phosphate solubilizing microorganisms to compare with control treatment. The result shows that the solubilizing index ranged between 1.4 to 1.6 for isolated microorganisms, the decrease in soil pH which have enormous influence in phosphate solubility were significantly influenced by the addition of phosphate solubilizing microorganisms and manure is 6.5 pH comparing with 8 pH for control. Soil soluble and extractable phosphorous also increased by combination of phosphate solubilizing microorganisms and manure recording 0.46±0.03 ppm and 10.7±1.01 ppm respectively comparing to 0.39±0.01 ppm and 0.75±0.11ppm for control after one month of experiment, and this significance continued to 3 and 5 month after planting followed by the combination treatment of rock phosphate and phosphate solubilizing microorganisms. Total phosphorous in both wheat shoot and root after 5 month of planting were increased significantly by the addition of phosphate solubilizing microorganisms and manure to record 0.792±0.11% in shoot and 0.66±0.13% in roots and more than other treatments, however the translocation of phosphorous is harder than nitrogen from roots to shoots. The addition of phosphate solubilizing microorganisms enhanced the growth ratio of wheat up to 96±5 comparing to 40±3 for control.

Key words: Phosphate solubilizing ,solubility , extractable ,fertilizer.

اداء الكائنات الحية الدقيقة المذيبة للفوسفات على السماد الحيواني والفوسفات الصخري وتاثيرها على فسفور التربة والنبات

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المستخلص

تم عزل الكائنات الحية الدقيقة المذيبة للفوسفات في التجربة المختبرية ، وزرع محصول القمح في الأصص لمدة 5 أشهر وعوملت بالفوسفات الصخري والسماد الحيواني مع معاملات تداخلية من الكائنات الحية الدقيقة المذيبة للفوسفات والفوسفات الصخري والسماد الحيواني لتقارن بمعاملة السيطرة . أظهرت النتائج أن مؤشر الذوبان تراوح بين 1.4 إلى 1.6 للكائنات الحية الدقيقة المعزولة ، وقد انخفض درجة حموضة التربة التي لها تأثير هائل في ذوبان الفوسفات بشكل كبير بإضافة الكائنات الحية الدقيقة المذيبة للفوسفات والسماد الحيواني ليصل pH 6.5 مقارنة مع pH 8 لمعاملة السيطرة. كما زاد الفوسفور القابل للذوبان في التربة والقابل للاستخلاص من خلال الجمع بين الكائنات الدقيقة المذيبة للفوسفات والسماد الحيواني وتسجيل ppm 0.46 ± 0.03 و ppm 10.7 ± 1.01 على التوالي مقارنة بـ ppm 0.39 ± 0.01 و ppm 0.75 ± 0.11 لمعاملة السيطرة بعد شهر واحد من التجربة ، واستمرت هذا التاثير بعد 3 و 5 أشهر من الزراعة يتبعها معاملة الفوسفات الصخري مع الكائنات الحية الدقيقة المذيبة للفوسفات. تمت زيادة الفوسفور الكلي في كل من المجموع الخضري والجذري للقمح بعد 5 أشهر من الزراعة بشكل كبير في معاملة الكائنات الدقيقة المذيبة للفوسفات والسماد الحيواني لتسجل 0.792 ± 0.11٪ في المجموع الخضري و 0.66 ± 0.13٪ في الجذور وأكثر من المعاملات الأخرى ، ولكن نقل الفوسفور أصعب من النيتروجين من الجذور إلى المجموع الخضري. عززت إضافة الكائنات الحية الدقيقة القابلة للذوبان في الفوسفات نسبة نمو القمح حتى 96 ± 5 مقارنة بـ 40 ± 3 مع معاملة التحكم.

الكلمات المفتاحية: السماد الحيواني، ذوبان، موضة، التربة، الفوسفات الصخري.

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INTRODUCTION

P is an essential and macronutrients just after nitrogen for plant growth and development. Plants absorb P from soil solution as orthophosphate as $HPO4_2^-$ and $H_2PO_4^-$. The solubility of orthophosphate is controlled by the pH of soil and by the presence of Ca^{+2},Mg^{+2}, Fe^{+3}, and Al^{+3}, orthophosphate are extremely reactive through precipitation with cations such as Ca^{2+}, Mg^{2+} in alkaline soils like calcareous soils dominated in Kurdistan which cultivated by wheat crop. And have the same problem in acidic soils through precipitation with cations such as Fe^{3+} and Al^{3+}, or adsorbed to Fe-oxides and Al-oxides, Al-silicates. In calcareous soils were Ca-carbonates are dominant depending on the particular properties of a soil like high soil pH and clay content, in these state, P is highly insoluble and unavailable to plants (26). Most agricultural soils are deficient in P content, rendering P one of the most important nutrients limiting agricultural production especially wheat crop. Soil microbial population is the key component of the soil that governs the almost all biochemical reaction in the soil including nutrient cycling, biocontrol agent, promoting plants growth and seedling (15 , 35 ,36). The productions of organic acids are the principle mechanism for mineral phosphate solubilization. Also alkaline and acid phosphatases enzyme play major role in the mineralization of organic phosphorus in soil (29). The most accepted mechanism of mineral phosphate solubilization is the action of organic acids produced by soil microorganism almost by fungi. Synthesis of organic acids leads to the acidification of the microbial cell and their surroundings (1) .It is well known that phosphorus in the soil is unavailable for plants, but the use of plant associated organisms may aid in mineral P solubilization for easy uptake by the plants. Fungi can solubilize P by production of organic acids and more efficiently than bacteria. Fungi also can promote plant growth by siderophore production. Arbuscular mycorrhizal fungi (AMF) are capable of Iron (Fe) uptake from associated host plants (16). Penicillium fungus is the key groups of soil microorganisms contributed in P cycling. (12 , 35). The global attitude now is focused on the use of bio fertilizer as phosphate solubilizing microorganisms as it was ecofriendly and cheap comparing with chemical phosphate fertilizers as super phosphate which deteriorate ecosystem. Releasing huge amounts of phosphate on water bodies and cause artificial eutrophication, contain trace amounts of heavy metal as Cd, and As, beside their effects on human being health, finally its high costs for farmers (5,23 , 31 , 35). Phosphate is highly insoluble and unavailable to plants. As the results, the amount available to plant is usually in a small proportion. It is generally accepted that the major mechanism of mineral phosphate solubilization is the action of organic acids synthesized by soil microorganism (11). The recovery of total P fertilizer applied is very low by wheat and make about 15–20% as it fixed by soil rendering it insoluble/ The only 0.1% soluble and available for plant uptake from the total P (2). Because calcareous soils that contain huge amount of $CaCO_3$ and distributed all over Kurdistan Region especially Duhok governorate and rendered phosphorous insoluble and wheat crops is cultivated wildly in a condition of rain feeding. So the aim of this research will focus on the effects of adding bio fertilizer of phosphate solubilizing microorganisms, rock phosphate and manure in phosphorous solubilization and availability over growth period of wheat.

MATERIALS ANDMETHODS

Isolation and growth conditions of P-solubilizing microorganisms:

phosphate solubilizing microorganisms strains were isolated from soil samples of Sumail district, Duhok province, Iraqi Kurdistan region. Soil samples (2 kg) were collected from the areas where wheat plants are cultivated by digging the ground at 0-15 cm depth, and stored at 4 °C in refrigerator. Each sample was added to 9 ml Dist. water with 10-fold dilution series. 0.1 ml dilution was purred on Pikovskaya agar (0.5 g $(NH_4)_2SO_4$, 0.5 g $MgSO_4.7H_2O$, 0.3 g NaCl, 0.3 g KCl, 0.03 g $FeSO_4.7H_2O$, 0.02 g $MnSO_4.H_2O$, 10.0 g $Ca_3(PO_4)_2$, 10.0 g Glucose and 15.0 g Agar) and plate count agar (5.0 g Tryptone, 2.5 g Yeast extract, 1.0 g Glucose and 15.0 g agar) by spread plate technique and incubated at 37 °C for 3-5 days. Various colonies on the 10-4, 10-5 and 10-6 dilution plates which showed clear zone indicating the ability for solubilizing tricalcium phosphate were selected and grown on Pikovskaya agar. The selected fungal strains were then re-streaked on the same medium (8, 32).

Fig. 1. The presence of clear zone around black colonies on right of plate indicates the ability of isolated Aspergillus sp. fungus to solubilize rock phosphate

Aspergillus sp is able to solubilize all sorts of unavailable phosphate as rock phosphate, aluminum phosphate and tricalcium phosphate (Kang, et al,. 2002) .Wheat crop were planted in pots containing 2 kg of calcareous soil and treated with manure 2% only, rock phosphate 2% only, manure with phosphate solubilizing fungi as liquid pikovskaya medium about 225 ml, Rock Phosphate with phosphate solubilizing fungi and some pots were leaved without treatments as control (6 , 17)..

Chemical analysis

The sodium bicarbonate ($NaHCO_3$) procedure of is generally accepted as a suitable index of P "availability" for alkaline soils; where the solubility of calcium phosphate is increased because of the precipitation of Ca as $CaCO_3$.For soluble P, the distilled water used instead of sodium bicarbonate and measured by ascorbic acid method (3). Total nitrogen in soil, plants shoot and root are determined using conc.H_2SO_4 as digestion material according to Kelhdjahel method. Soil pH and electric conductivity by pH meter and all parameters were analyzed after 1, 3 and 5 month from planting.

Phosphorus solubilizing activity

Screening for primary phosphate solubilizing activity of the isolates was carried out by allowing the fungi to grow in selective media, i.e., Pikovskaya's agar for media for 7 to 10 days at 25°C. The appearance of a transparent halo zone around the fungal colony indicated the phosphate solubilizing activity of the fungus. And the solubilizing index calculated according to this equation. Solubilizing index = Colony diameter + clearing zone / Colony diameter

Statistical analysis

The data has been analyzed by repeated measures ANOVA with general linear model (GLM) procedure to determine the significant effect ($P \leq 0.05$) of treatments on studied parameters over time. Data were analyzed by a series of analyses of variance. Subsequently, Tukey's HSD (Honestly Significant Difference) test was used to identify significant differences between treatment means and changes in tested parameters over time. All statistical analyses were carried out using the Minitab software package 17.

RESULTS AND DISCUSSION

Figure 2 and Table 1 indicate that the Manure+ PSM has the most excellence effects on the growth ratio of wheat plant then Rock P+ PSM, that mean the PSM has significant role in excreting induced exudates to promote plant growth. From the tables 1,2,3 and figure 1 it also obvious that the PSM has significant role in increasing both soluble and available phosphorous from rock phosphate and manure, also promote vegetative dense and plant flourishing in most cases. Solubilizing index ranged between 1.4 and 1.6 for isolated fungi (Kang et al. K.D. 2002).

Fig. 2. Wheat treated with Manure+ PSM in right shows more vegetative and growth ratio than treated with Rock P+ PSM in the middle comparing with control in the left

Table1.Wheat growth Ratio in various treatments

Control	Manure	Manure+ PSM	Rock P	Rock P+ PSM
40±3	64±4	96±5	54±3	76±4

From table 2, ANOVA and P - value obviously shows that the PSM has a tremendous effects on the release and solubility of available P forms both extractable and soluble as well as in decreasing soil pH that control the solubility of P. The P-solubilizing activity of the PSM comes from biochemical synthesis and release of organic acids, to chelate cations like calcium in calcareous soils which bound to phosphate through their hydroxylic and carboxylic groups and render it in soluble forms (Kpomblekou & Tabatabai, 1994). While PSM release low molecular weight organic acids on mineral rock phosphate by certain strains of PSB (Goldstein 1995) (Kim et al. 1997)(Sheikh-Abdullah & et al. 2019).

Table 2. Summary of ANOVA (General Linear Model) for measures of Soluble P, Extractable P and pH. Effects treatments, Time and their interactions

	treatments		Time		Treatments × Time	
	F4,30	P - value	F2,30	P - value	F8,30	P - value
soluble P	231.85	<0.0001	12.91	<0.0001	17.59	<0.0001
Extractable P	12840.99	<0.0001	57.23	<0.0001	51.57	<0.0001
pH	97.87	<0.0002	30.2	<0.0002	29.28	<0.0002

Table 3 shows statistical analysis of various treatments on soil phosphorous form and pH as well as plant content of nitrogen and phosphorous both in shoots and roots after five month planting. Result revealed tremendous effects of PSM on rendering phosphorous available in soil and plants absorption. The treatment of Manure + PSM significantly increased the amount of both soluble and extractable phosphorous as well as the total amount of both nitrogen and phosphorous in the plant root, while in shoots the translocation of phosphorous is seem to be harder than nitrogen from roots to shoots. soil pH is also significantly decreased by the presence of phosphate solubilizing microorganisms because P dissolving bacteria are well known to reduce the pH of the surrounding soil by the excretion of organic acids such as acetic, formic, lactic, propionic, fumaric, glycolic, and succinic acids which easily dissociated and release a considerable amounts of hydrogen ions which acidify the medium (Barea 2000; Barea et al. 2002; Chalot et al. 2002) (Issazadeh L. et al, 2018). The treatment of Rock P + PSM ranked the second on its positives effects on studied parameters. The PSM are superior in making phosphorous more soluble because phosphorous availability is related to the number and activity of soil dwelling microorganisms especially fungi. And microbial biomass and manure contains a huge amounts of P, which in return available to plants, fungi considered a super agents in the dissolution of phosphates from both organic and inorganic sources (Barea 2000; Barea et al. 2002; Chalot et al. 2002).

Table 3. effect of treatments on studied soil and plants parameters. For N in root, N in shoot, P in root and P in shoot the analysis were performed on the data obtained at the end of the experiments (Month five).

Treatments	Soil Soluble P ppm	Soil Extractable P ppm	pH	N Root %	N shoot %	P root %	P shoot %
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Control	0.328 ± 0.05d	0.848 ± 0.08e	7.81 ± 0.26a	0.557 ± 0.03d	1.171 ±0.06c	0.447 ± 0.04b	0.557 ± 0.15a
Manure	0.447 ± 0.03b	10.333 ± 0.41c	7.33 ± 0.50c	0.840 ± 0.02b	1.792 ± 0.01b	0.654 ± 0.07ab	0.563 ± 0.07a
Manure + PSM	0.516 ± 0.04a	11.533 ± 0.78a	6.92 ± 0.30e	1.171 ± 0.05a	2.161 ± 0.05a	0.796 ± 0.20a	0.657 ± 0.12a
Rock P	0.329 ± 0.03d	9.456 ± 0.21d	7.50 ± 0.08b	0.673 ± 0.02c	2.100 ± 0.12a	0.570 ± 0.09ab	0.650 ± 0.12a
Rock P + PSM	0.419 ± 0.02c	10.556 ± 0.32b	7.14 ± 0.16d	0.677 ± 0.01c	1.790 ± 0.03b	0.572 ± 0.13ab	0.545 ± 0.20a
P-value	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.05	> 0.05

Means that do not share letter in a column are significantly different at probability level ($P \leq 0.05$).

Table 4 shows the statistical analysis of soil soluble P, extractable P and pH changes after 1,3,and 5 month after wheat planting in plastic pods in different treatments. Result revealed that soil soluble P reached its peak after one month then declined slightly with time, whereas soil extractable P take 3 month to reach its highest amounts indicating that the positive effects of PSM in P availability last for 5 months. The positive effects of PSM in soil pH showed decreasing in pH in the whole time of experiment and the peak decline were noticed after 5 month. There are well known inverse relationship between the soil pH and soluble phosphorous concentration documented by many researchers that the PSB strains via production of organic acid decrease soil pH through the acidification of the soil medium and enhancing the solubilization of phosphorous (Illmer and Schinner 1995) and (Hwangbo et al. 2003) (Issazadeh et al. 2018).

Table 4. Changes in soil soluble P, Extractable P and pH during different time period

Months	Soil Soluble P ppm	Soil Extractable P ppm	pH
	Mean ± SD	Mean ± SD	Mean ± SD
1	0.441 ± 0.04a	8.34 ± 3.96c	7.38 ± 0.5a
3	0.420 ± 0.10a	8.79 ± 4.18a	7.47 ± 0.37a
5	0.391 ± 0.09b	8.50 ± 4.04b	7.18 ± 0.35b
		Summary of ANOVA (P –Value)	
	<0.0001	<0.0001	<0.0001

Means that do not share letter in a column are significantly different at probability level (P ≤ 0.05).

Table 5 shows the treatments of soil by manure, rock P and PSM and there interaction effects with time on the soil soluble P ppm, extractable P ppm and soil pH. The treatment of Manure+ PSM gives best effects on soil solubilize and extractable after 3 and 5 month, then manure treatment gives the second best effects on releasing soluble and extractable phosphorous also after 3 month. This effect of PSM and manure is higher in the case of extractable phosphorous from low to adequate range in soil according worldwide standards. Soil pH adversely affected by adding PSM and manure and highest decrease in pH were obtained in Manure+ PSM after 1 month treatment, then in manure after 5 month, then in Rock P+ PSM also after 5 months (Omar 1998) (Bayan and Umer 2018) (Fokin et al 2014a).

Table 5. interaction effect of the treatments and time on the Soil soluble P ppm, extractable P ppm and pH.

		Months 1	3	5
		Mean ± SD	Mean ± SD	Mean ± SD
Soil Soluble P ppm	Control	0.39 ± 0.2ef	0.31 ± 0.015gh	0.28 ± 0.02h
	Manure	0.42 ± 0.01de	0.48 ± 0.015bc	0.45 ± 0.015cd
	Manure+ PSM	0.47 ± 0.01c	0.56 ± 0.015a	0.52 ± 0.02ab
	Rock P	0.36 ± 0.01fg	0.32 ± 0.02gh	0.31 ± 0.015h
	Rock P+ PSM	0.42 ± 0.01de	0.43 ± 0.03cde	0.40 ± 0.005def
Soil Extractable P ppm	Control	0.75 ± 0.006i	0.95 ± 0.015i	0.84 ± 0.012i
	Manure	9.9 ± 0.1ef	10.27 ± 0.058d	10.83 ± 0.115c
	Manure+ PSM	10.7 ± 0.2c	12.47 ± 0.058a	11.43 ± 0.058b
	Rock P	9.63 ± 0.058fg	9.5 ± 0.2gh	9.23 ± 0.116h
	Rock P+ PSM	10.73 ± 0.058c	10.77 ± 0.058c	10.17 ± 0.252de
pH	Control	8.00 ± 0.10a	7.95 ± 0.05a	7.49 ± 0.14bcd
	Manure	7.56 ± 0.09bc	7.75 ± 0.096ab	6.68 ± 0.052gh
	Manure+ PSM	6.57 ± 0.116h	7.00 ± 0.05f	7.19 ± 0.177def
	Rock P	7.50 ± 0.1bcd	7.47 ± 0.115bcde	7.55 ± 0.099bc
	Rock P+ PSM	7.27 ± 0.153cdef	7.16 ± 0.2ef	6.98 ± 0.136fg

Means that do not share letter among five treatments and time are significantly different at probability level (P ≤ 0.05).

Table 6 revealed the effects of each treatment over time on the changes of soil soluble P ppm, extractable P ppm, and soil pH. The time has significant effects even in control treatment and soil soluble P released at its peaks after 1 month from wheat planting reflecting the maximum effects of indigenous soil PSM which need one month to release most soluble P while it need about 2 month to release most extractable P and significantly decreased soil pH over first three months of growing. Manure alone has an excellent influence in rendering P available till five months due to its richness by essential nutrients and release of wide range of organic acids so significantly decreased soil pH continuously till five months so the extractable P reached it high after 5 month (Krey et al. 2013). Manure+ PSM treatment considered the supreme in all aspect's including time and maximum available P lasted for three months and conserved optimum soil pH to the end of growing season due to a harmonized mixture of Manure+ PSM in liberating available forms of P through the whole 5 months. The addition of rock phosphorous alone don't make a distinct differences in both soluble and extractable P over control treatment as the release of these forms is quite difficult than its liberation from organic manure that enhance the flourish of indigenous PSM more than rock P.The addition of PSM to rock P improved the solubility of rock P till 5 month and aids in a significant release of extractable P during the third beginning month of wheat growing (Kimet al. 1997)-- (Saber et al 2009) (Fokin et al 2014b) (Fokin et al 2014c). Soil pH also decreased about one unit comparing to control during the whole period of growing season (Idrees & Ismail 2019).

Table 6. changes in Soil Soluble P ppm, extractable P ppm, and pH under each treatments over time

Control		Soluble P ppm	Extractable P ppm	pH
	months	Mean ± SD	Mean ± SD	Mean ± SD
	1	0.39 ± 0.02a	0.75 ± 0.006c	8.00 ± 0.1a
	3	0.31 ± 0.015b	0.95 ± 0.015a	7.95 ± 0.05a
	5	0.28 ± 0.02b	0.84 ± 0.0116b	7.49 ± 0.14b
	P - value	0.001	< 0.0001	0.002
Manure	1	0.42 ± 0.012b	9.9 ± 0.1c	7.56 ± 0.09a
	3	0.48 ± 0.015a	10.27 ± 0.058b	7.75 ± 0.096a
	5	0.45 ± 0.015ab	10.83 ± 0.115a	6.68 ± 0.052b
	P - value	0.006	< 0.0001	< 0.0001
		Mean ± SD	Mean ± SD	Mean ± SD
	1	0.47 ± 0.01b	10.7 ± 0.2c	6.57 ± 0.116b
Manure+ PSM	3	0.56 ± 0.015a	12.47 ± 0.058a	7.00 ± 0.05a
	5	0.52 ± 0.02a	11.43 ± 0.058c	7.19 ± 0.178a
	P - value	0.001	<0.0001	0.002
		Mean ± SD	Mean ± SD	Mean ± SD
	1	0.36 ± 0.01a	9.63 ± 0.06a	7.50 ± 0.1a
Rock P	3	0.32 ± 0.02b	9.5 ± 0.2ab	7.47 ± 0.01a
	5	0.31 ± 0.012b	9.23 ± 0.12b	7.55 ± 0.1a
	P - value	0.01	0.031	0.52
Rock P+ PSM		Mean ± SD	Mean ± SD	Mean ± SD
	1	0.42 ± 0.01a	10.73 ± 0.058a	7.27 ± 0.153a
	3	0.43 ± 0.03a	10.77 ± 0.058a	7.16 ± 0.02a
	5	0.40 ± 0.01a	10.17 ± 0.252b	6.99 ± 0.136a
	P - value	0.15	0.005	0.067

Means that do not share letter in each column and for each separated treatments are significantly different at probability level ($P \leq 0.05$).

CONCLUSIONS

PSM with manure and Rock P have the most excellence effects on the growth ratio of wheat plant by excreting induced exudates to promote plant growth and ensure the growth of wheat seeds. PSM with manure and Rock P have tremendous effects in the release and solubility of available P forms both extractable and soluble as well as in decreasing soil pH that control the solubility of P. PSM with manure and Rock P have significantly increased the amount of both soluble and extractable phosphorous as well as the total amount of both nitrogen and phosphorous in the plant root, while in shoots the translocation of phosphorous is seem to be harder than nitrogen from roots to shoots. The addition of PSM and manure combined or alone to the soil ensured a decrease in soil pH for one unit that indicates the liberation of available phosphorous in calcareous soils. The use of PSM and manure combined or alone ensured an adequate range of available phosphorous in calcareous soils at least for 3 months which has well known problems with phosphorous availability. So it recommended adding PSM and manure combined or alone to the soil at least one time in the annual season of wheat growing.

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